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Some Enumerative Studies on Malarial Fever.

BY MAJOR RONALD ROSS, F.R.S., AND DAVID THOMSON, M.B., CH.B., D.P.H.

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Some Enumerative Studies on Malarial Fever.

By Major RONALD ROSS, F.R.S., and DAVID THOMSON, M.B., Ch.B., D.P.H.

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Prefatory Note by R. Ross.—Towards the end of last year the Advisory Committee for the Tropical Diseases Research Fund (Colonial Office) allotted considerable funds to the Liverpool School of Tropical Medicine for researches to be carried out in Liverpool. This enabled us to commence, under my direction, a number of minute co-ordinated studies on cases admitted into the Tropical Ward of the Royal Southern Hospital—material which, though it offered peculiar facilities for research, had long remained neglected owing to want of funds. The cases (occurring in the clinics of Dr. Macalister, Dr. Lloyd Roberts, and myself) were placed in charge of Dr. David Thomson; the chemical studies in charge of Dr. G. C. E. Simpson; the parasitological studies in charge of Dr. H. B. Fantham. Parallel researches on animals were also assigned to Dr. John Thomson who is working under Sir Edward Durning-Lawrence's fund for the investigation of the effect of temperature on disease; Dr. V. T. Korke (Research Fellow) has studied coagulation times and other details; the literature was in charge of Mr. W. R. Drawz, the Malaria Bibliographer (Advisory Committee's Fund); and much valuable help has been given by the staffs of the University, the School of Tropical Medicine, and the Royal Southern Hospital of Liverpool, and by Sister Linaker of the Tropical Ward.

The researches were commenced on January 1, 1910. A paper by Dr. David Thomson and myself, describing a regular periodical increase of the trypanosomes in a case of Sleeping Sickness, was published;* and we now present to the Society brief accounts of our results regarding malaria, blackwater fever, trypanosomiasis, and various therapeutical agencies, obtained (mostly by new methods) up to the end of July, 1910. Further details will be published, if necessary, in the 'Annals of Tropical Medicine,' Liverpool.

1. *Preliminary.*—For many years past little information which is both new and exact has been added to our knowledge of the pathology of malaria. This has probably been due to the exhaustion of the older methods of research, which, being purely qualitative, have failed to indicate the precise correlations between the numbers of the parasites present in a patient and the various pathological and therapeutical reactions. For example, out of

* 'Roy. Soc. Proc.,' B, July 21, 1910, vol. 82.

51 and 38 successful inoculations of men by means of infected blood and infected Anophelines respectively, in not a single one has any exact estimate been given of the numbers of parasites inoculated, or recovered after the lapse of the incubation period; and, though many researches on quinine have been made, we know of none in which its direct effect upon the numbers of the parasites in the patient has been correctly measured. Moreover, the older methods often failed to reveal the parasites at all, unless they were present in large numbers. Hence our first care was to elaborate more exact methods both for detection and for enumeration. This was done early in the year (by R. R. and D. T.)—though we have by no means reached finality yet. The new methods were next employed for all the cases in the Tropical Ward, daily counts of the parasites and often of the leucocytes, together with other estimations, being made (by D. T.). At the same time parallel chemical studies were carried out (by G. C. E. S.) and therapeutical ones (by D. T.); and the measurements were carefully charted and compared. Even at this preliminary stage the results (which are given at the end of this paper) include more accurate verifications of some old conjectures, and demonstrations of some new theorems.

2. *The Detective Method Used.*—In the ordinary methods the blood is spread out in a thin film, liquid or dry—1 cu. mm. covering, say, 4 sq. cm. of area. If the field of the oil-immersion lens has a diameter of 0·165 mm., and can be thoroughly searched by the eye in three seconds, about 12 hours' work would be required to examine the whole of 1 cu. mm. of blood in this way; and if the specimen contains only one parasite it may not, by bad luck, be found until nearly the whole of the area has been searched. We have, therefore, employed the "thick film process" described by one of us (R. R.) in 1903. In this 1 cu. mm. of blood is spread out over only about one quarter of a square centimetre or less, and dried; the hæmoglobin is then gently washed out with water; and the residue, consisting of parasites, leucocytes, platelets, and the stromata of the red corpuscles, is carefully stained by any convenient method, with or without fixation. By this simple means the whole of 1 cu. mm. of blood can be searched in less than an hour; but the identification of the Plasmodia requires considerable practice.

3. *The Enumerative Method Used.*—The younger Plasmodia are too small to be counted by an ordinary hæmocytometer; hence their number has usually been estimated by comparison with the number of red or white cells, the latter figure being determined by the hæmocytometer. This gives a large compound error; for if e is the percentage error made in counting the red or white cells in one specimen, and e' is the percentage error made in

computing the proportion of parasites to each corpuscle in that specimen, then, by a simple calculation, the total percentage error will be $e+e'+ee'/100$. (For example, if $e = +5$ per cent. and $e' = +10$ per cent., the total percentage error will be $+15{\cdot}5$ per cent.)

Our method consists simply in making a measured quantity of blood into a thick film preparation and then counting *all* the parasites in it. The blood is measured by determining the diameter of a fine capillary tube under the microscope and then calculating and marking off the length of the tube required to hold the given quantity (as suggested by Dr. Wakelin Barratt). Thus a uniform tube of 0·180 mm. diameter and 4 cm. length will contain 1·018 cu. mm.—a convenient size. The blood is sucked up into the tube and then quickly discharged upon the glass slide in one or several measured droplets, first samples being rejected. The dehæmoglobinised thick film is fixed before staining, in order to avoid loss, and the whole area is scrupulously searched with the aid of a sliding stage.

Mr. M. Greenwood, Junior, informs us that if n is the number of parasites counted in the unit (say, 1 cu. mm.) of blood, and m is the number of such units in the patient's whole body, then the total number of parasites in him, assuming uniform distribution, will be $mn \pm 0{\cdot}67449m\sqrt{n}$. Thus the probable percentage error is $67{\cdot}449/\sqrt{n}$, the factor m cancelling out from the ratio. This is important, because it shows that the error depends, not on the magnitude of the measured sample taken, but upon the number of parasites actually counted in it. Hence, in order to obtain results within an assigned error, we take a large sample when the parasites are scarce and a small one when they are numerous; the number of parasites which we must find and count being given by the formula $n = 4550/e^2$, where e is the permissible error.

The greatest error occurs when the parasites are so scarce that it is difficult to find enough of them to count up to the required standard; or when they are so numerous that it is difficult to measure a volume of blood small enough to contain an easily countable number of them. In these preliminary researches we have seldom examined at one sitting more than 1 cu. mm. of blood, or less than $\frac{1}{4}$ cu. mm.

When the parasites were very numerous, we have sometimes fallen back on estimating their number by comparison with leucocytes, but are now elaborating improvements and special instruments for such, and other, details. The method is also accurate and rapid for other blood parasites, and for leucocytes. Other sources of error are (1) inaccurate measurements of the quantity of blood used, and (2) difficulty of seeing or distinguishing badly stained parasites.

One kilogramme of blood of average density (1057·5) contains 945,626 cu. mm., and the blood in a man's body is estimated to weigh about 4·9 per cent. of the total body weight—from which data it is easy to compute the total number of parasites in a patient. A man of 64·74 kilogrammes, or about 10 stone, will contain about 3,000,000 cu. mm. of blood.

4. *The Cases studied* by us numbered 33. All had been infected in West Africa or America, so that none was of less than some weeks' duration when admitted into hospital in Liverpool. All were males of from 18 to 60 years of age. They were mostly shipmen or traders, and two were negroes. The parasites of all were counted almost every day and sometimes several times a day ; and the temperature was taken every four hours, or more often. One case showed *Plasmodium malariæ*, together with crescents (sexual forms of *P. falciparum*). Eight showed *P. vivax* only, and 24 *P. falciparum* only, two of the latter containing only sexual forms. Quinine was often withheld for some days while other methods of treatment were used. The cases were studied for over 600 days altogether. There were no deaths.

All the cases will be recorded in the 'Annals of Tropical Medicine,' vol. 4, No. 3, but charts of Cases 17 and 20 are given on pp. 164 and 165.

At this preliminary stage of the enquiry we have undertaken to study only the gross daily correlations between the numbers of parasites and other phenomena—leaving more minute hourly or four-hourly analysis to future work. Hence, if a number of counts or other observations have been made on one day, we have recorded only the averages in the tables. For temperatures, however, it has been thought best to record maxima instead of averages, and, as the tables should be as economical as possible, we have used the *hæmatothermic scale* suggested by one of us (R. R.), in which the 10° between 95° and 105° F., or between 35° and 45° C., are divided into 100 parts. Except in Case 6, specially studied by Dr. Fantham, we have not distinguished in the tables the various forms, sets, or stages of *P. vivax*—this being reserved for future study. Similarly, the treatment, leucocytes, hæmoglobin, and urobilin are not differentiated too minutely. Case 25 was counted by Dr. Korke.

5. *The Correlation between the Parasites and the Fever.*—It is well known that fever is caused only by the asexual forms of the parasites, but though these are generally thought to be more abundant during pyrexial periods, no extensive numerical studies on the point appear to have been made, with the result that some sceptics still profess doubts on the subject. Our cases, except four without fever (Cases 1, 15, 16, 27), would seem to indicate a very strong (almost convincing) correlation. Twenty-one of them suffered each from one pyrexial period, lasting from one to seven days, and preceded

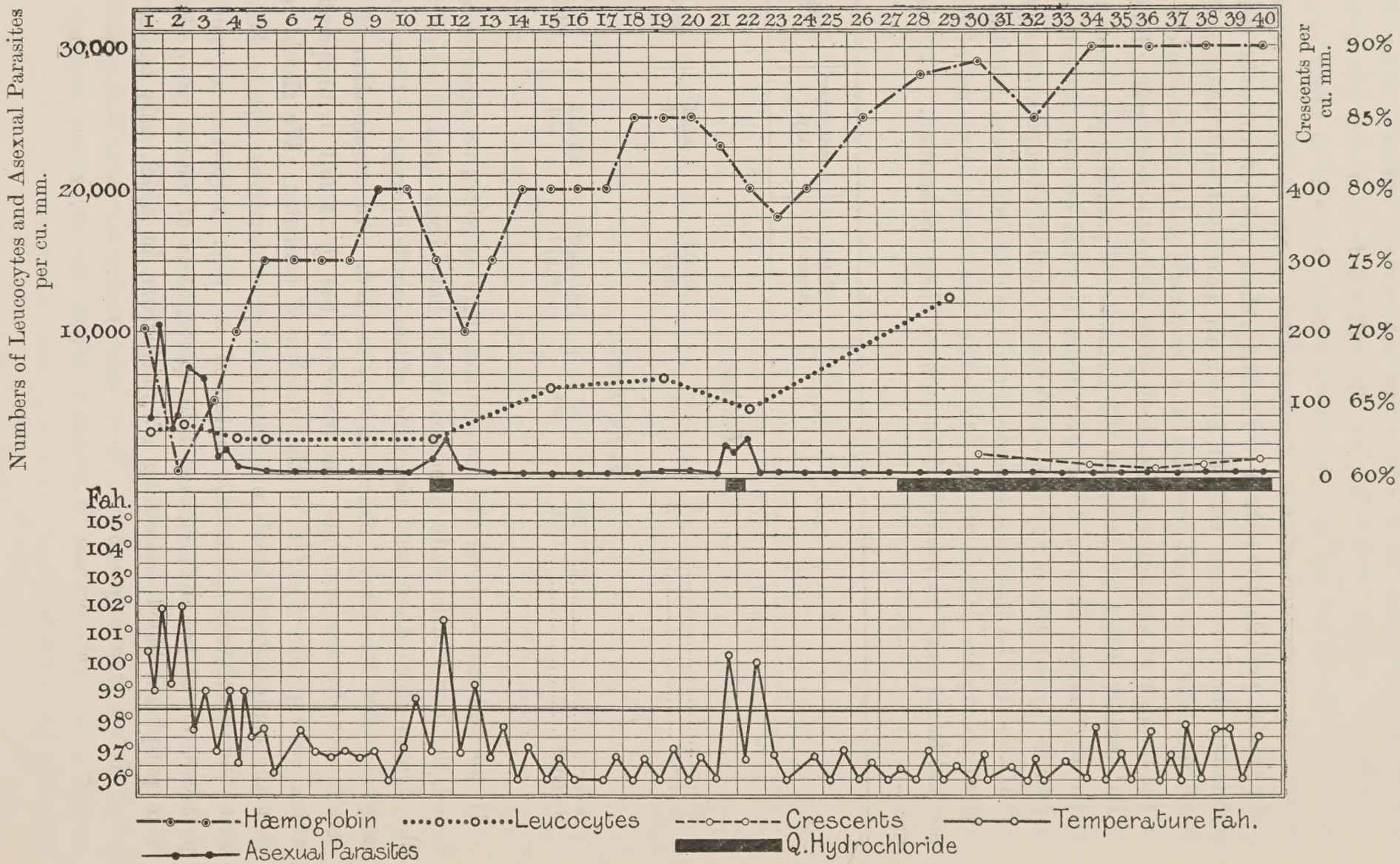
or followed by apyrexia, and in all, except Case 32, the asexual parasites were very much more numerous during the pyrexial period. Nine of the cases (7, 17, 18, 19, 20, 23, 24, 26, 32) suffered from one or two relapses each (13 relapses altogether), and every relapse, as well as every original attack, was associated with a comparatively large number of parasites (43 pyrexial periods altogether). Similarly, there were 46 apyrexial periods (including the four cases without fever), all associated with comparatively small numbers of asexual parasites, and conversely not a single marked rise in the number of asexual parasites took place without corresponding fever.

For further detail we compare the numbers of parasites per cubic millimetre counted on days of fever with those counted on days without fever. A febrile day is taken as any one on which the patient's temperature exceeds 98°·6 F. (= 36° hæmatometric scale), or any one which comes between two tertian paroxysms (Cases 9, 14, 24). The non-febrile days on which no asexual forms could be found in 1 cu. mm. of blood are omitted. The results are—

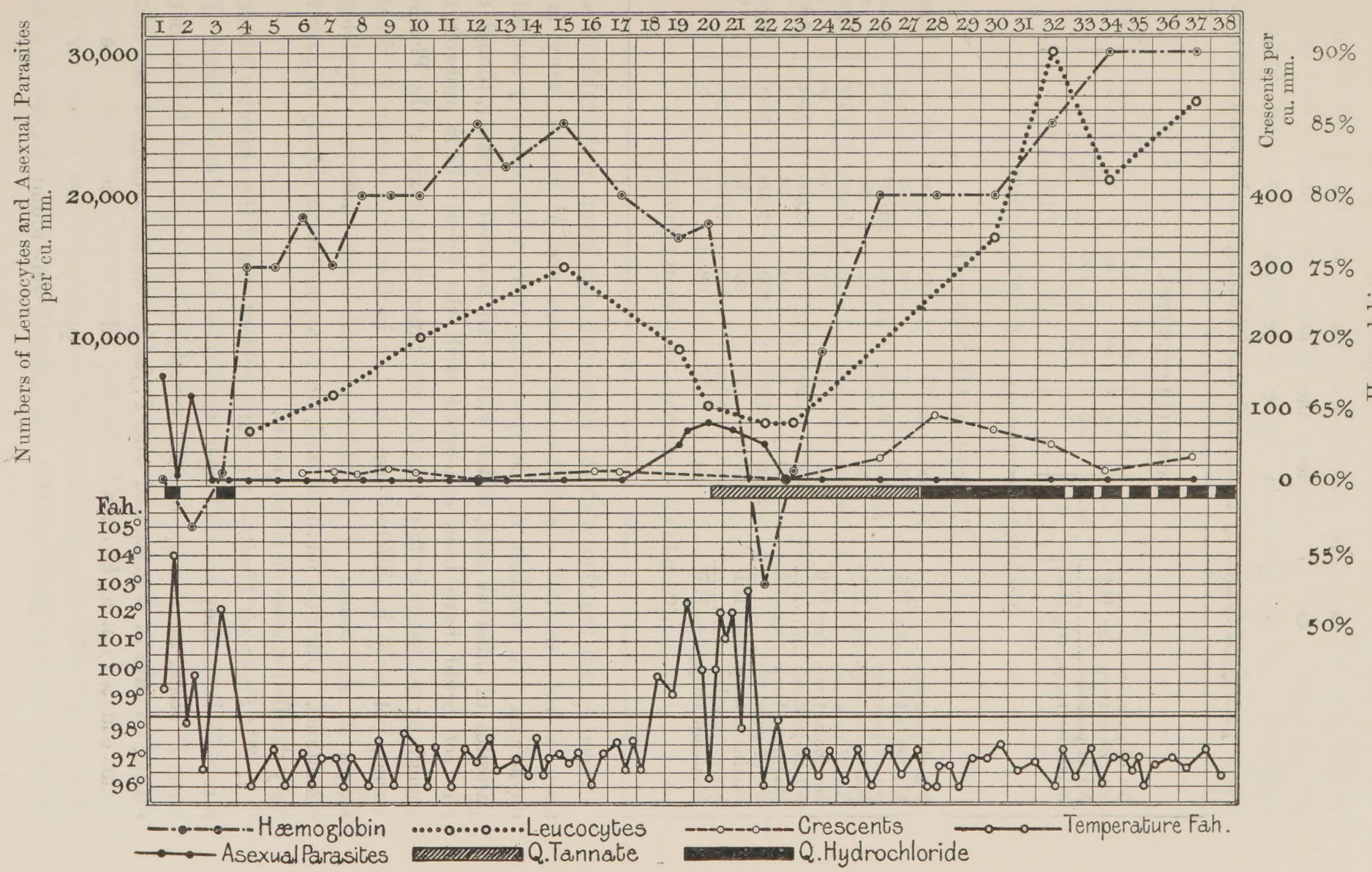
	Cases.	Days.	Total parasites.	Average per day.	Ratio.
			P. vivax.		
Fever	8	30	136,062	4,535	36·3
No fever	8	68	8,487	125	
			P. falciparum.		
Fever	21	97	1,319,880	13,607	29·6
No fever	19	83	38,277	461	

If we had not omitted the non-febrile days on which no parasites were found, the non-febrile daily averages would have been much smaller than the figures given above, and the ratio of the febrile averages to them much larger, so that the febrile excess is very marked. An examination of the details will further convince the reader of the great fall in temperature which accompanies the fall in the number of parasites. We should note that the comparatively small numbers of *P. vivax* found are due to the fact that the specimens generally contained a large proportion of mature parasites, not as yet disintegrated into spores, these of course being absent from peripheral blood containing *P. falciparum*, in which only spores or young forms generally occur. There is no reason for considering quinine in this connection, because it probably affects the fever only through its action on the parasites.

CASE 17.—R.B. *P. falciparum.*



CASE 20.—F.B. *P. falciparum.*



6. *The Degree of Fever Associated with Various Numbers of Parasites.*—We know of no extensive and exact studies on this point, and ours are not yet complete. On 47 occasions the number of parasites was carefully compared with the maximum degree of temperature reached during the febrile paroxysm caused by them, and the following results were obtained:—

P. vivax (12 occasions).

No. of parasites	1500	1400	580	440	430	260	256
Maximum temp., deg. H.F.	84	101	97	102	76	76	54
No. of parasites	232	150	133	100	83		
Maximum temp., deg. H.F.	46	44	34	34	20 (rigor)		

(Coefficient of correlation), $r = 0 \cdot 68561 \pm 0 \cdot 10318$.

P. falciparum (35 occasions).

No. of parasites	303,000	58,000	55,000	54,000	50,000	45,000
Maximum temp., deg. H.F.	108	82	88	100	98	76
No. of parasites	36,000	34,000	31,000	26,000	26,000	25,000
Maximum temp., deg. H.F.	60	86	76	94	101	80
No. of parasites	16,000	15,000	15,000	15,000	9,700	9,000
Maximum temp., deg. H.F.	52	54	54	70	56	70
No. of parasites	7,500	7,400	6,700	6,500	6,000	5,800
Maximum temp., deg. H.F.	90	40	86	83	52	70
No. of parasites	5,200	4,000	4,000	3,600	3,000	2,600
Maximum temp., deg. H.F.	76	70	40	80	38	64
No. of parasites	2,500	2,000	1,860	1,000	200	
Maximum temp., deg. H.F.	50	46	38	15	50	

(Coefficient of correlation), $r = 0 \cdot 60331 \pm 0 \cdot 07251$.

These figures, together with the numerous ones given in the table of cases, show a very marked correlation, but there are, of course, many minor deviations. In numbers of the specimens there were certainly two sets of parasites of different ages, which were often counted together, though only one set could have been concerned with the fever. It is probable also, though by no means certain, that the resistance to the toxin of the Plasmodia varies, not only in different persons, but in the same person at different stages in the course of his infection and under different physiological conditions. Our new methods will, we hope, enable us shortly to undertake very detailed studies on these points.

7. *The Pyrogenic Limit.*—It is quite evident that if the parasites fall below a certain limit, they are no longer numerous enough to cause fever. Thus with *P. vivax*, an average of 125 parasites of all ages and forms counted during 68 days in 8 cases, and with *P. falciparum* an average of 460 asexual forms counted during 83 days in 19 cases, failed to produce fever. The actual limit above which they become pyrogenic probably varies in different cases With *P. vivax*, as many as 1500, 852, and 540 were found in Cases 2, 6, and 7

without fever, and as few as 150 and 50 in Cases 5 and 9 with very slight fever (98°·6 F.), so that about 200 to 500 may perhaps be taken as the usual limit. With *P. falciparum*, as many as 1620 (asexual) were found as an average of four non-febrile days in Case 26, and 1196 as an average of six non-febrile days in Case 25; while in Case 18 from 16 to 1860 were present for 22 days without causing any marked fever, so that 600 to 1500 may perhaps be adopted as the usual limit. With *P. malariæ* (Case 1), an average of 79 were present for 13 days without fever, but about 140 twice caused slight rigor.

8. *The Asexual Forms between the Relapses.*—It is generally thought and taught that these forms tend to "disappear" between the relapses—though of course exceptions, especially in children, are noted—and to reappear during them; and several hypotheses, such as that of parthenogenesis (F. Schaudinn), have been advanced to explain the supposed phenomenon, and have led to what appears to be much waste of work. We find that the asexual forms do not necessarily disappear at all between the relapses, but are generally still to be found in small numbers per cubic millimetre on most of the days. The apyretic periods preceding the 13 relapses in our cases (7, 17, 18, 19, 20, 23, 24, 26, 32) lasted 114 days altogether (nearly nine days each on the average). On 18 of these days no observations were made; but on the remaining 96 days the parasites were found in small numbers on 59, or 61 per cent., and were not found only on 37. Moreover, the general trend of the curves suggests that they were not found on these days only because their numbers were a little too few for detection. The parasite curve, at its height during a pyrexial period, generally falls very rapidly at first and more slowly later, and tends to reach its lowest about half-way between two apyrexial periods. At this point it may or may not remain above the detectable limit (by thick-film methods). After this it was observed (by D. T.), especially in Cases 7, 17, 23, and 24, to begin mounting slowly at first, until, when it reached the pyrogenic limit, another pyrexial period commenced. All this is scarcely compatible with the speculation that the apyrexial periods are due to the *abrupt* death of most of the asexual Plasmodia, or to their conversion into "resting stages," etc. Nor do such speculations appear to be at all necessary. It is easy to see that the survival of comparatively small numbers of the asexual forms will suffice to keep the infection alive, not only for the short periods observed by us, but for "relapses of long interval," and for months or years. Obviously, if only a few parasites per cubic millimetre are present, they may easily be overlooked in the small amount of blood (say 0·1 cu. mm.) usually examined by thin film, and may then be reported as being absent: yet many millions may still exist

in a patient who contains, say, 3,000,000 cu. mm. of blood. The speculation regarding parthenogenesis in malaria, and the case of Schaudinn supposed to support it, have been criticised elsewhere by one of us (R. R.).* In two of our relapsing cases (19, 26), no sexual forms at all were found during 35 different thick-film examinations. Even if they were present in small numbers (crescents do not collect in the spleen), yet the numbers of asexual parasites found in or just before the relapses (24 and 400 per cu. mm.) cannot be explained by parthenogenesis unless we suppose that each sexual form produced by "gametoschizogony" 840 or 14,000 spores! Until better evidence for such views is adduced, our results justify the doctrine that the malarial infection is kept continuously alive simply by the persistence of the asexual forms in varying numbers, and that fever occurs only when the forms are numerous enough to produce it.

9. *The Effect of Quinine on the Asexual Forms.*—The destructive effect of the drug is, of course, everywhere recognised, though very little completely satisfactory statistical evidence can be cited in proof. Many laborious researches have been made regarding the comparative utility of the various salts, but these have been confined almost entirely to estimating the rate and percentage of absorption, judged from urinary elimination. The subject is of the greatest sanitary and medical importance; but, so far as we can see, it can be usefully studied only by the more detailed enumerative analysis which we propose soon to undertake. Our daily analysis gives a little more light; but the following figures may be mentioned. Cases 15 and 16 showed no asexual forms; Cases 11 and 13 were so severe that quinine was given at once; Cases 1, 5, 27, 31 and 32 were so mild that quinine was withheld during the period of observation; and in the remaining cases the drug was withheld only for some days (except an occasional necessary dose), and was then given continuously while the parasites were still being counted. We have therefore added together all the daily counts during the periods of no-quinine and quinine respectively. With eight cases of *P. vivax* there were 45 no-quinine days, showing 104,032 total parasites, or an average of 2,312 a day; and there were 74 quinine days, with 31,769 total parasites, or an average of only 429 a day (one-fifth). With *P. falciparum* (19 cases), 147 no-quinine days showed an average of 3,136 asexual forms, and 148 quinine days an average of 1,535 (one-half). We should have expected that the proportion of quinine-day parasites would have been smaller; but the no-quinine counts are diminished by the inclusion of the five mild cases and also by doses of 10 to 20 grains which were given on 10 isolated occasions. On quinine days the doses were generally

* 'Prevention of Malaria' (Murray, 1910).

20 to 30 grains a day, and Cases 11, 13, 14, 15 and 16, and parts of other cases are omitted because they were unsuitable. Great falls in the number of parasites occurred, even without quinine, in Cases 5, 17 and 26; while, on the other hand, small numbers of asexual forms remained in some cases for five or more days in spite of considerable daily dosage. In Case 23 a severe relapse began six days after the stoppage of quinine tannate, which had been given for nine previous days in 10 to 30 grain doses daily. No other relapses occurred after continuous quinine treatment; but 12 occurred without it.

10. *Observations on the Sexual Forms.*—It is, of course, generally held—and probably quite rightly—that these are developed (by a cytological process not yet clearly seen) from the asexual forms. But, at the same time, no one has been able to note any correspondence between their numbers—many of the former may be present when the latter are very scarce, and *vice versâ*. This rule, which is fully confirmed by our figures, has always been difficult to reconcile with the accepted theory of origin. On comparing our curves, however, the remarkable fact was observed (by D. T.) that there often seems to be indeed a correspondence, but that it is delayed for from 8 to 10 days—that is, that the sexual curve tends to rise that number of days after a rise in the asexual curve. Out of our 25 infections with *P. falciparum* 18 showed crescents, and of these Cases 14, 22, 17, 18, 20, 23, 24 and 30 suggest this phenomena, while in 17 and 20, the rises are sufficiently isolated to show it most distinctly. In Case 17, asexual rises existed on the 1st to 3rd days, the 11th to 12th and the 21st to 22nd days, separated by apyrexial periods during which few asexual and no sexual forms were found; but on the 30th day (eight days after the last relapse) crescents appeared for the first time. In Case 20, an asexual relapse commenced on the 17th day and reached its height on the 20th day. A very few crescents had been found all the time; but a marked rise in their numbers commenced on the 26th day and reached its height on the 28th day. The most ready explanation is that the *crescents require 8 to 10 days for development*, during which they remain undetected, probably in the internal organs of the host. This is confirmed by the fact that numerous small ones are seen at the commencement of a rise; and also by the observation (frequently made) that in fresh infections the crescents seldom appear until a week or more after the first attack of fever. Strong confirmation is also given by Case 6, in which Dr. Fantham noted a sudden appearance of sexual forms (*P. vivax*) on the 11th day, 7 to 10 days after the original asexual rise, and after the patient had been taking 30 grains of quinine daily for a week, suggesting that the same phenomenon holds for *P. vivax* also.

Other observations are as follows:—(1) By no means every asexual rise is followed by a sexual one. In seven cases (13, 19, 25, 26, 28, 31, 33), no crescents at all were seen. In Case 13, a negro, 173,400 asexual forms per cubic millimetre failed to produce any; and no crescents were observed in another negro (Case 28). (2) The number of crescents never exceeded 5 per cent. of asexual forms found in the generation which (hypothetically) produced them, and were generally in a much smaller proportion. (3) We could find no relation to youth, vigour, amount of hæmoglobin, duration of infection (from 3 to 30 weeks), or season of first infection. (4) The daily counts of crescents generally show marked daily variations, but we think that after considerable doses of quinine have been given for some days these daily variations tend to be smoothed out (Cases 16, 18, 22, 24). (5) In Cases 15, 14 and 16 particularly, a distinct tertian tendency was observed in the daily variations of crescents. This appears not to have been previously noticed, but is just what would be expected in the case of a tertian parasite; though, of course, the curve would be confused in a double tertian. (6) Sometimes the crescent curve suddenly rises with great rapidity, reaches its maximum, and then begins to fall on the next day with equal rapidity; though, later, the fall often tends to be much more slow (Cases 14, 15, 22, 23), especially when the numbers are very small. Apparently, the greater the maximum the quicker the fall (Cases 14, 15); and the fall is sometimes jagged, with a tertian tendency (*ibid.*). (7) Sometimes the maximum is irregularly maintained for a number of days (Cases 16, 18, 24), especially when quinine has not been previously given. (8) The crescents remained detectable for as many as 31, 32, 32, 35 and 44 days in Cases 20, 22, 23, 24 and 18 respectively; and finally disappeared (in 1 cu. mm. of blood) in Cases 1, 11, 12, 16, 21 and 23.

From these data we think (1) that a varying percentage of asexual forms are constantly generating crescents, which, after about 8 to 10 days, appear in the peripheral blood. Hence, when the asexual forms remain sufficiently numerous for a period, as when no quinine is given, the stock of crescents is being constantly replenished. Thus the mortality among the older crescents is constantly being compensated for by new arrivals, and the total numbers appear to remain constant; an appearance which has given rise to the generally accepted hypothesis (perhaps erroneous) that the crescents survive for weeks. On the other hand, the sudden rise and fall of their numbers seen in Cases 14, 15, 22 and 23 suggest that they really survive only for a short time after their appearance in the peripheral blood. In Cases 16 and 24, however, a few crescents were still found after quinine had been given continuously for 16 to 18 days, suggesting that some of them

may be able to survive for longer periods. In Cases 20, 22 and 23, they appeared in increasing numbers for 4 to 6 days after continuous 30-grain doses of quinine had been commenced, suggesting that the drug has no effect upon them when they have once been generated. But quinine appears to affect the numbers of crescents indirectly by cutting off the source of supply. Though many researches upon the effect of quinine on crescents (a subject of the greatest sanitary importance) have been attempted, so far as we know, the possibility of a constantly continued supply has not previously been taken into consideration. We are continuing researches on the point.

11. *The Leucocytes* were counted frequently, but not always daily; at first by the hæmocytometer, but in the later 20 cases by the thick-film method. The latter method has the advantages of putting the leucocytes closer together and of enabling us to estimate them at the same time with the parasites; but, like the hæmocytometer, it is not very appropriate for differential counts, because of occasional uncertainty in distinguishing the various kinds. Accordingly, these were made (in six cases) by thin film, 500 cells being examined on each occasion.

Our results are, as shown in all the cases, that during the pyrexial periods the total leucocytes are comparatively few, being frequently as low as 2000 to 3000 per cubic millimetre (Cases 17, 18, 23, 24, etc.). But as the asexual forms and the fever diminish, the total leucocyte curve rises, and about seven days later exceeds the normal, and may reach 20,000 and even, rarely, 30,000 per cubic millimetre (Case 7)—though this height is not maintained. The very high counts seem to be associated with much quinine. We observed generally that the so-called polymorphonuclear leucocytes, though rather few during the pyrexial periods, do not vary much from day to day, but about 7 days after the fever has abated their numbers are markedly increased and vary much. The so-called mononuclear percentage was always in excess throughout the cases, even long after the disappearance of all forms of the parasites—for six weeks afterwards in Case 1, though quinine had been given daily during that period (out of hospital). We think, therefore, that a high mononuclear percentage is likely to be always of value in diagnosis in the absence of parasites. The normal ratio of total mononuclears to polymorphonuclears is about 35 per cent., but in our cases it was often 60 per cent, and in Case 30 reached 80 per cent. It tends to fall slightly with great improvement in health. During the individual daily paroxysms, as shown by Stephens and Christophers, the total mononuclears are rather low during the height of the fever, but show a marked increase during the following remission. This increase is chiefly due to the large mononuclears. This process is repeated with each paroxysm, and if a paroxysm fails the

mononuclears remain (Case 9 especially). But we could not find these variations in the absence of fever and many parasites. This mononuclear reaction, which we have also seen in our case of trypanosomiasis, appears to be exactly comparable to the polymorphonuclear reaction described by F. W. Andrewes* as generally occurring in bacterial diseases.

12. *Various Therapeutic Agents.*—Methylene blue was tried in three cases in 12-grain daily doses; the parasites diminished, but we cannot draw any conclusions from the figures. In Case 24, altogether 36 grains of soamine were injected intramuscularly in 5 to 10 grain doses on five occasions during 12 days, and yet a smart relapse occurred during and after the last dose. In the same case, just before the same relapse, X-rays had been applied over the spleen and abdomen for 20 minutes. The asexual parasite rise had commenced before the application. In Case 24, faradic and galvanic currents were applied over the spleen, each for 10 minutes, but crescent counts made immediately before and two hours after the application remained unaltered.

13. *Hæmoglobin.*—This was estimated in most of the later cases. The percentage always fell during fever, and began to rise rapidly shortly afterwards, proving the destructive effect of the parasites and the rapid recuperation of the patients. The greatest fall was 25 per cent. after four days' fever (Case 20); but more detailed work is required to trace correspondence with the number of parasites. The lowest percentage found was 53 per cent. All the patients were anæmic at first, but the hæmoglobin rose rapidly with improvement of health, and very rapidly in the more vigorous subjects. We decided not to attempt counts of red corpuscles, as these have been made so frequently already. The elimination of the hæmoglobin and the findings in a case of blackwater fever will be dealt with in the following paper by Dr. Simpson and in one by ourselves.†

14. *Summary.*—(1) There is a very decided correlation between the number of asexual Plasmodia found in the peripheral blood and the fever.

(2) As a rule, no fever exists unless the asexual forms exceed some hundreds per cubic millimetre.

(3) The asexual forms do not always disappear between relapses (as often thought) but tend to persist in small numbers per cubic millimetre, and often increase again for some days before the actual febrile relapse occurs.

(4) These observations give a coherent theory of the malarial invasion, according to which the infection is kept alive indefinitely by the ordinary sporulation of the asexual forms, and not by parthenogenesis or by resistant

* 'Lancet,' June, July, 1910.

† 'Annals of Tropical Medicine,' Liverpool, vol. 4, No. 3.

forms; and fever recurs only when the parasites are numerous enough to produce it.

(5) We estimate from our cases that considerable continued doses of quinine reduced the asexual forms by 50 to 80 per cent.

(6) There are strong reasons for supposing that the sexual forms require eight to ten days for development; that the often noticed long persistence of crescents is not due to their long life (as generally thought) but to constant replenishment of the stock by fresh broods; that they sometimes show a distinct tertian periodicity; and that quinine does not affect them when once generated, but ultimately reduces their numbers by destroying the generating cells. The sexual forms were never seen to produce fever.

(7) The leucocytes are below normal during febrile periods and above normal afterwards. The percentage of mononuclears rises greatly after paroxysms, and is always in excess of the normal.

(8) Methylene blue, soamine, X-rays, and faradic and galvanic currents had no results in a few experiments.

(9) The hæmoglobin falls markedly with fever, but rises rapidly with convalescence.

(10) The fæcal urobilin shows marked correlation with the occurrence of fever, and is specially studied in the accompanying paper by Dr. G. C. E. Simpson.*

Many other deductions may be based on our observations, but will be better considered after more detailed counts have been made.

* 'Roy. Soc. Proc.,' B, vol. 83, p. 174.

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